Moringa oleifera ameliorates lipid metabolic disorders, oxidative stress and the inflammatory status in high fat diet-induced obesity in rats

Samy A. Hussein*, Yakout A. El-Senosi, Ghada, H. EL-Sharkawy

Department of Biochemistry, Faculty of Vet. Med., Benha University, Egypt.

* Corresponding author: Samy A. Hussein, e-mail: samyaziza@yahoo.com

ABSTRACT
Moringa oleifera leaves have therapeutic functions such as antioxidant, anti-bacterial, anti-viral, anti-cancer, anti-inflammatory, anti-allergic and anti-diabetic. The purpose of this study was to evaluate the protective effect of Moringa oleifera on obesity induced oxidative stress and biochemical abnormalities in male rats. Forty-eight male albino rats were divided into three equal groups. Group I (control normal): rats fed normal diet. Group II (Obesity): rats received high fat diet (HFD). Group III (Obesity + Moringa oleifera): rats received Moringa oleifera (600 mg/kg.b.wt.) orally for two months after induction of obesity. The obtained results showed significant increase in levels of serum insulin, lipids profile (total cholesterol and triacylglycerols), liver L-MDA in addition to up regulation of Leptin and Nuclear factor kappa B (NF-κB) gene expression level in obese rats. However, liver catalase activity and GSH concentration were markedly decreased. Moringa oleifera treatment to high fat diet-induced obesity in rats caused significant improvement of all previous parameters towards its normal ranges. These results suggested that, Moringa oleifera treatment exerts a protective effect on obesity by reduction of oxidative stress markers, inflammation and hyperlipidemia in rats through free radical scavenging and anti-inflammatory activities as well as regenerating endogenous antioxidant defense system mechanisms.

Keywords: Obesity, Moringa oleifera, oxidative stress, leptin, NF-κB.

1. INTRODUCTION
Obesity is the weight of a particular individual taken in kilograms divided by the height taken in the square [1]. The causes include excessive intake of calories, physical inactivity, Sedentary lifestyles, urbanization, endocrine disorders, irregular metabolism, lack of sleeping time, medications, eating patterns, inheritance and environmental [2]. According to the Pharmacological Management of Obesity: An Endocrine Society Clinical Practice (ESCP) Guideline”, recommended by the National Heart, Lung, and Blood Institute [3] the most ideal treatment modality for weight loss should be appropriate dietary and lifestyle changes plus moderate-intensity exercise. However, many epidemic and clinical studies have shown that it is a great challenge to maintain long-term lifestyle modification [4]. Thus, natural supplements products primarily helping consumers to fight the battle against obesity have been widely explored. A variety of natural plants (e.g., herbs, fruits, and vegetables), functional fatty acids (e.g., polyunsaturated fatty acids and conjugated fatty acids), and other natural dietary compounds have been used in different anti-obesity products. Natural plant products are expected to be potential ingredients for the development of nature-sourced anti-obesity products in the weight loss segment due to rising consumer health awareness [5].

Recently, a great number of natural plants have shown to be considered as new approach to combat obesity and associated liver disease. The bioactive compounds that are responsible for relieving such stress are believed to be polyphenols and flavonoids compounds as Moringa plant [6].

Moringa is called a kind of magical healthy plant, which is rich in nutrients, and also contains Moringa polysaccharides, flavonoids, c-amino butyric acid and...
other active ingredients, with good therapeutic care function; so it is known as the Magic Tree and Tree of Life [7].

Various preparations of M. oleifera exhibited antibiotic, hypotensive, anti-ulcer, anti-inflammatory and anti-cancer properties [8]. The edible leaves of M. oleifera tree have been known as an anti-diabetic food for centuries [9]. The aqueous extract of M. oleifera leaves demonstrated potent antioxidant and antidiabetic activity [10]. Furthermore, the methanolic extract of M. oleifera leaves improved dyslipidemia and body weight gain in experimentally induced obesity in rats [11]. Recently, extract of M. oleifera leaves has been shown to have hypcholesterolemic and antioxidant activities in obese rats [12]. Accordingly, this study was undertaken to evaluate the possible beneficial effect and antioxidant activity of Moringa oleifera against deleterious effect of obesity in adult male rats through investigation of insulin, lipid profile, oxidative stress markers in addition to Leptin and NF-κB gene expression.

2. MATERIALS AND METHODS

2.1 Experimental animals:
Forty-eighth white male albino rats of 12-16 weeks old age and average body weight 160-200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. Rats were fed on constant ration and fresh, clean drinking water was supplied ad libitum. All rats were acclimatized for minimum period of 15 days prior to the beginning of study.

2.2 Ration and additives:
There are two type of ration were prepared freshly and daily throughout the course of experiment: a standard diet with protein 20%; fat 5%;carbohydrates 5%; fiber 5% and a high-fat diet with 20% of energy derived from protein;15% from corn oil; 50% from sucrose; 5% from fiber [13].

2.3 Induction of obesity:
The experimental induction of obesity in male rats was induced by feeding the rats on the prepared high fat diet (HFD) for eight weeks before the beginning of the experiment. The diet was prepared and necessary vitamins and minerals were added. For fatty diet the chow, in powder form, was mixed fat until become homogenous in a dough-like consistency. This dough was shaped with a paste injector. The obtained chow blocks were dried and used for rats feeding for two months. Two months after obesity induction, treatment with Moringa Oleifera were given and continued for additional two months.

2.4 Chemicals and antioxidants:
The antioxidant and chemicals used in the present study were:
- Moringa oleifera was purchased from National Research Institute, Egypt.

Moringa oleifera were dissolved in distilled water, freshly prepared and administered orally and daily at a dose level of 600 mg/kg body weight for group III for two months [14].

b- Other chemicals used in this study were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt.

2.5 Experimental design:
After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (10 rats each) placed in individual cages and classified as follow:
- Group I (normal control group): Rats fed normal diet, received no drugs, served as control non- treated for all experimental groups.
- Group II (Obesity induced group): Rats received high fat diet (HFD) ad libitum, served as obesity induced rats group.
- Group III (Obesity + Moringa oleifera treated group) rats received Moringa Oleifera orally at a dose of (600 mg/kg,b.wt./day) for 2 months after induction of obesity.

2.6 Sampling:
Blood samples and liver tissue specimen were collected from all animal groups (control and experimental groups) once after the end of 4 months.

2.6.1 Blood samples:
Blood samples were collected by ocular vein puncture from all animal groups after overnight fasting in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic pippte and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: Insulin, Total cholesterol and Triacylglycerols.

2.6.2 Tissue samples:
2.6.2.1 Liver tissue for biochemical analysis
About 0.5 g of liver tissue specimen was taken from each group of rats after had been euthanized. The specimens were immediately removed and washed several times with saline and blotted between two damp filter papers, weighed and stored at -20°C for subsequent biochemical analyses.

2.6.2.2 Preparation of liver tissue homogenate:
Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: L-MDA and Catalase.
About 0.2 g of liver tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clear supernatant was removed and used for determination of GSH concentration.

2.6.2.3 Liver tissue for molecular analysis:
About 0.5 of liver tissue put in eppendorf tubes and were immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of Leptin and NF-κB gene expression level.

2.6.3 Biochemical analysis
Serum Insulin was determined according to the method described by Wilson and miles, (1977)[15]. Total cholesterol and Triacylglycerols were determined according to the method described by NCEP expert panel, (1988)[16] and Stein, (1987)[17]; respectively. Liver tissue L-MDA, CAT and GSH were determined according to the method described by Mesbah et al., (2004)[18], Luck, (1974)[19] and Moron et al., (1979)[20].

**Molecular analysis:**
Total RNA was isolated from liver tissue of rats using RNeasy Mini Kit (Thermo Qiagen, #74104) according to the manufacturer’s protocol. Following determination of RNA concentration and purity by Quawell nanodrop Q5000 (USA), 5 mg of total RNA from each sample was reverse transcribed using Quantscript reverse transcriptase. The produced cDNA was used as a template to determine the relative expression of Leptin and NF-κB genes using Step One Plus real time PCR system (Applied Biosystem, USA) and gene specific primers. The reference gene, β-actin, was used to calculate fold change in target genes expression. The thermal cycling conditions, melting curves temperatures, and calculation of relative expression was done. For the treated groups, assessment of $2^{-ΔΔCT}$ determined the fold change in gene expression relative to the control [21].

### Table 1: Effect of *Moringa oleifera* administration on serum insulin, total cholesterol and triacylglycerol concentrations in obesity induced in male rats.

<table>
<thead>
<tr>
<th>Exp. groups</th>
<th>Insulin (µU/ml)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Normal control</td>
<td>4.68± 0.21 a</td>
<td>80.21± 2.53 a</td>
<td>57.15± 3.83 a</td>
</tr>
<tr>
<td>Group II: (Obese)</td>
<td>9.82± 0.37 b</td>
<td>155.50± 3.85 b</td>
<td>113.00± 4.63 b</td>
</tr>
<tr>
<td>Group III: Obese + <em>M. Oleifera</em></td>
<td>5.73± 0.22 c</td>
<td>107.11± 2.47 c</td>
<td>86.90± 3.15 c</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E).  S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

### Table 2: Effect of *Moringa oleifera* administration on liver tissue L-MDA, Catalase and GSH in obesity induced in male rats.

http://ijps.aizeonpublishers.net/content/2019/2/ijps2045-2052.pdf

---

3. RESULTS AND DISCUSSION

3.1 Effect of *Moringa Oleifera* administration on serum insulin, total cholesterol and triacylglycerol concentrations in obesity induced in male rats:
The data presented in table (1) showed a significant increase in serum insulin, total cholesterol and Triacylglycerol concentrations in obesity-induced rats when compared to normal control group. However, *Moringa Oleifera* treatment to obese male rats caused a significant decrease in elevated serum insulin, total cholesterol and Triacylglycerol concentrations when compared with obesity induced group.
3.2 Effect of *Moringa oleifera* administration on liver tissue L-MDA, Catalase and GSH in obesity induced in male rats:
The obtained results presented in table (2) revealed that, obese rats showed significant increase in liver tissue L-MDA and significant decrease in Enzymatic antioxidant catalase activity and Non-enzymatic antioxidant reduced glutathione (GSH) when compared with normal control group. On the other hand, *Moringa oleifera* treatment to obese male rats caused a significant decrease in liver tissue L-MDA with marked increase in catalase activity and GSH concentration when compared with in obesity–induced rats.

3.3 Effect of *Moringa oleifera* administration on relative expression of Leptin and NF-κβ gene level in liver of obesity induced in male rats:
The qPCR results demonstrated in table (3) showed a significant up-regulation in relative expression of Leptin and NF-κβ gene level in liver tissue of obesity induced rats when compared to normal control group. However, *Moringa oleifera* treatment to obese rats caused a significant down-regulation in Leptin and NF-κβ gene expression level when compared with obesity–induced rats.

Cardiovascular disease, including heart disease, is becoming crisis and major cause of morbidity, mortality [22] and disability and premature death throughout the world and it substantially contributes to the escalating costs of health care. In most cases, these clinical conditions result from atherosclerosis, which was once identified as a lipid-storage disease [23]. These observed effects of obesity are largely mediated through: hormonal imbalance and a cluster of metabolic disorders including dyslipidemia [24], and glucose intolerance [25] which, in turn, elevate risk of cardiovascular diseases. Other variables including, oxidative stress, inflammation and hematological changes can also be considered risk factors for increasing Cardiovascular disease in obesity. The obtained results demonstrated that, obesity–induced rats showed significant increase in serum insulin, total cholesterol and triacylglycerol concentrations when compared to normal control group. These results are nearly similar to Woods et al.,(2004) [26] who recorded that, hyperinsulinemia and insulin resistance are induced by high-fat feeding. Also, Cyrus et al., (2003) [27] showed that, mice fed HFD had a significant increase in both cholesterol and triglycerides, as well triglycerides level was higher in the high fat fed rat groups and lead to the development of abnormal lipid metabolism and atherosclerosis [28]. Insulin resistance in obesity is also characterized by chronic low-grade inflammation and reduced plasma levels of adiponectin, an adipokine that improves insulin sensitivity. Along with increased plasma levels of free fatty acids, hyperglycemia, and reactive oxygen species, these factors can change gene expression and cell signaling in vascular endothelium which also alters
the release of endothelial vasoactive factors [29]. Loss of insulin action causes a shift in balance from oxidation to esterification of free fatty acids (FFAs), resulting in elevated very low-density lipoprotein (VLDL) secretion. Also, insulin resistance can be considered as additional factor which contributes to increased cardiovascular disease in obesity. The action of insulin is initiated by binding to its receptors and activation of intrinsic protein tyrosine kinase activity of the receptors, resulting in initiation of intracellular signaling cascade that eventually related to glucose and lipid metabolism [30]. It is well established that increased availability and utilization of free fatty acids (FFAs) play a critical role in the development of insulin resistance. Excess adipose tissue has been shown to release an increased amount of FFAs which directly affect insulin signaling, diminish glucose uptake in muscles, drive exaggerated triglyceride synthesis and induce gluconeogenesis in the liver leading to elevated levels of glucose and lipids [31].

Treatment with *Moringa oleifera* to obese male rats caused a significant decrease in serum insulin, total cholesterol and triacylglycerol concentrations in. These results were nearly similar to those recorded by Waterman et al., (2015) [32] who found that, the uses of moringa as a dietary agent in preventing type 2 diabetes (T2D) in mice fed very high fat diet + 3.3% Moringa caused significant reduction in weight gain, hepatic adiposity, gluconeogenesis, insulin, cholesterol and inflammatory markers; and increase in insulin signaling sensitivity and lipolysis as moringa exert their effects by inhibiting rate-limiting steps in liver gluconeogenesis resulting in direct or indirect increase in insulin signaling and sensitivity and that suggest that moringa may be an effective dietary food for the prevention and treatment of obesity and type 2 diabetes.

The current study revealed that, obese rats showed significant increase in liver tissue L-MDA and marked decrease in catalase and GSH when compared to normal control group. Similarly, Balkan et al., (2002) [33] reported that, high-cholesterol (HC) diet had an increasing effect on lipid peroxidation in plasma and tissue in rabbits. High fat diet (HFD) has a role in generating oxidative stress, which results from an imbalance between the production of free radicals, and the scavenger antioxidant system [34]. Obesity is also associated with oxidative stress, which may be due to extended postprandial hyperlipidemia and/or hyperglycemia. Obese humans have increased levels of oxidative stress and this is ameliorated by diet restriction and weight loss [35]. Also, obesity is associated with increased activities of the secondary product of lipid peroxidation, and weight loss has positive effects on oxidative stress and antioxidant activity [36]. Also, increased lipid profile could also contribute to increased oxidative stress in obesity, where increased lipid substrate in the tissues may increase the mechanical and metabolic load on such tissues, thus increasing oxygen consumption. A negative consequence of the elevated oxygen consumption is the production of reactive oxygen species (ROS) [37]. Lipid peroxidation is a marker of cellular damage initiated by ROS. In obesity, lipid peroxidation is thought to play a role in the etiology of existing health problems, such as cardiovascular disorders. Increased lipid peroxidation is considered responsible for impairment of endothelial cells, capillary permeability and vascular integrity [38]. Further increase in superoxide, $\text{H}_2\text{O}_2$, hydroxyl radicals and lipid peroxidation consequently cause decrease in the effectiveness of antioxidant defenses, like SOD, CAT and GPx [39].

Treatment with *Moringa oleifera* to obese male rats caused a significant decrease in liver tissue L-MDA and markedly increased liver catalase activity and GSH concentration when compared to obesity induced group. These results are nearly similar to Mahmoud et al., (2017) [40] who demonstrated that, potent antioxidant activities of *M. oleifera* declined the observed rise in MDA level, and improved the reduction in GSH content in rats fed the HCD for a long period. These results are consistent with those reported by, Xu et al., (2004) [41] and Metais (2015) [42] who recorded that, the pronounced antioxidant effects of *M. oleifera* since they exhibited significant inhibition of lipid peroxidation induced by leptin resistance in obese rats liver homogenate and improved the antioxidant status via prevention of oxidative stress. Also, Ebrahim-Mameghani et al., (2016) [43] explained that, tocopherols and other antioxidants in Moringa oleifera has a significant protective activity against oxidative damage and play a central role along with fatty acids in decreasing the MDA level. This is suggested to occur by suppression of reactive oxygen species formation, the inhibition of enzymes or chelating of elements involved in the free radical production, scavenge reactive species, and upregulate antioxidant defenses. Moreover, Nutritional antioxidants such as vitamins A, C, and E provide additional protection from the stress [44]. Oxidative stress is widely accepted as a major contributing factor in the pathogenesis of CVD and diabetes [45]. Among the major classes of phytochemicals found in the plant, flavonoids appear to carry most of this activity. Flavonoids, which are synthesized in the plant as a response to microbial infections, have a benzo-γ-pyrone ring as a common structure [46]. Intake of flavonoids has been shown to protect against chronic diseases associated with oxidative stress, including cardiovascular disease and cancer. *M. oleifera* leaves are a good source of flavonoids [47]. A recurring explanation for the therapeutic actions of *M. oleifera* medication is the relatively high antioxidant activity of its leaves, flowers, and seeds [48]. It has hypolipidemic, hypotensive, and anti-diabetic properties in obese Zucker rats with metabolic syndrome [49]. It can reduce hyperlipidemia and atherosclerosis in high cholesterol or high-fat fed rabbits [50]. Also it can protect insulin- producing pancreatic B cells from Streptozotocin (STZ) induced oxidative stress and
apoptosis in rats. The beta carotene found in M. oleifera leaves has been shown to act as antioxidants. The antioxidants have the maximum effect on the damage caused by free radicals only when they are ingested in combination. A combination of antioxidants found in MO leaves was proven to be more effective than a single antioxidant, possibly due to synergistic mechanisms and increased antioxidant cascade mechanisms [51] and also exhibited high free radical scavenging activity. The extract of M. oleifera leaves also contains tannins, ascorbic acid, saponins, flavonoids, terpenoids and glycosides, which have medicinal properties. These compounds have been shown to be effective antioxidants, antimicrobial and anti-carcinogenic agents [52].

The Presented data indicated that, a significant up-regulation in relative expression of Leptin and NF-κB gene level were observed in liver tissue of obesity induced rats. These results are nearly similar to Tulipano et al., (2004) [53] who showed that, leptin level was higher in adult male Sprague-Dawley rats fed a high-fat diet (31% of energy) for one week. Most obese individuals have high concentrations of leptin but exhibit leptin resistance because of decreased leptin transport into the central nervous system or down regulation of leptin receptors [54]. Leptin communicates the amount of stored energy to the brain and activates the hypothalamic center which regulates energy intake and in turn body energy expenditure [14]. The amount of stored triglycerides in the adipose tissue correlates to the expression of leptin and circulating leptin concentrations [55]. There is provide evidence that, leptin was elevated in obese human [56] and animals [57]. Moreover, Masoud et al., (2006) [58] reported that, serum leptin concentration was increased in relation to increased body fat content. The positive correlation between body fat and serum leptin is probably explained by the increased release of leptin from large fat cells. Thus, leptin can serve as an indicator of fat content and its level may be decreased by reduction of body weight. Additionally, Lin et al., (2000) [59] suggested that, during high fat feeding animals are sensitive to the food lowering effect of leptin. However, despite the reduction in food intake, animals become fat as a result of the increase in food efficiency leading to an increase in leptin level followed by resistance to its action. Leptin is a cytokine like polypeptide produced by the adipocytes and it is overproduced during obesity due to the generation of ROS [60]. Inflammation is a host defense mechanism to protect against pathogens, stresses and tissue damage, and is a major factor in the progression of many chronic diseases including ulcerative colitis, diabetes, atherosclerosis, obesity and arthritis [61]. The inflammatory response to foreign pathogens and general stressful insults involves a combination of different signaling elements such as cytokines, nitric oxide (NO) and two key transcription factors, nuclear factor-kappa B (NF-κB) and nuclear factor (erythroid-derived 2)-like2 (Nrf2) is activated in most cell types as mice-fed HFD displayed about 3.5-fold increased whole body NF-κB [62]. Also, [63] reported that, NF-κB is increased about twofold in the liver, hypothalamus and skin of rodents fed with HFD for 6 months compared with animals fed with a control diet. NF-κB was similarly elevated in the liver and skin in common genetic obesity models of genetic hyperphagia (ob/ob mice and fa/fa rats) [64].

Moringa oleifera treatment to obese rats caused a significant down-regulation in Leptin and NF-κB gene expression level when compared with obesity–induced rats. These results are nearly similar to those recorded by Ahmed et al., (2014) [12] who showed that, the treatment of obese group with the extract of Moringa oleifera elicited significant decrease in leptin level. Moringa leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids [65]. Therefore, Moringa oleifera has the ability to scavenge free radicals with consequent inhibition of leptin level in serum as there is a significant positive correlation between leptin concentration and ROS generation [25]. Additionally, extracts of M. oleifera leaves restored body weight towards normal status and mechanistically declined dyslipidemia, and insulin resistance through its action on the gene expression of leptin, resistin as well as adiponectin of the visceral adipose mass. Therefore, the extract of M. oleifera leaves ameliorated hyperleptinemia, hyperresistinemia as well as hyperadiponectinemia in obese hypercholesterolemic rats [14]. Moringa oleifera are known for their antioxidant and anti-inflammatory effects [66], which are likely mediated through the activation of nuclear factor (erythroid-derived 2)-like2 (Nrf2) and inhibition of NF-κB, as it play a role in the prevention and treatment of chronic inflammatory conditions. Thus [67] showed that, Moringa oleifera showed inhibition of all the inflammatory markers the greatest inhibition was observed in iNOS expression/NO production, which may be a primary target for Moringa oleifera anti-inflammatory effects. The expression of these inflammatory mediators are activated by NF-κB, however they can be differentially regulated by other transcription factors [68]. As Moringa oleifera displayed strong anti-inflammatory and antioxidant properties in vivo and in vitro, making them promising botanical leads for the mitigation of inflammatory-mediated chronic disorders.

4. CONCLUSION
The present study demonstrated that, administration of Moringa oleifera relieved harmful effects caused by high fat diets induced obesity. Obesity affected different organs mainly liver and these occurred through changes in several parameters. High fat diets induced obesity caused significant increase in serum Insulin, total cholesterol, triacylglycerol and liver tissue L-MDA, Leptin and NF-κB. Conversely, a significant decrease in liver tissue Catalase and GSH were observed in obese rats. Moringa oleifera treatment in obese rats attenuates all previous parameters towards
its normal range. So, these results confirmed the strong antioxidant, anti-inflammatory effects of Moringa oleifera in obesity.

5. REFERENCES


40. Mahmoud, A., Metwally, F., Rashad, H. et al., (2017). The Potential of Moringa oleifera to Induce Cerebral Leptin mRNA Expression and to Attenuate Oxidative Stress, Cognitive and Motor Deficits, Depression- and Anxiety-


© 2019; AIZEON Publishers; All Rights Reserved

This is an Open Access article distributed under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.